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Standard Test Method for Enterococci in Water Using Enterolert™[1]

This standard is issued under the fixed designation D 6503; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method describes a simple procedure for the detection of enterococci in water and wastewater. It is based on IDEXX's patented Defined Substrate Technology® (DST™). This product, Enterolert, utilizes a nutrient indicator that fluoresces when metabolized. It can detect these bacteria at one colony forming unit (CFU)/100 mL within 24 h. The presence of this microorganism in water is an indication of fecal contamination and the possible presence of enteric pathogens.

1.2 This test method can be used successfully with drinking water, source water, recreational (fresh and marine) water, and bottled water. It is the user's responsibility to ensure the validity of this test method for waters of untested matrices.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

D 1129 Terminology Relating to Water[2]
D 1193 Specification for Reagent Water[2]
D 3370 Practices for Sampling Water from Closed Conduits[2]

3. Terminology

3.1 *Definitions*—For definitions of terms used in this test method, refer to Terminology D 1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *enterococci, n*—a gram positive bacteria possessing the enzyme β-D-glucosidase, which cleaves the nutrient indicator and produces fluorescence under a long wave length (366 nm) ultraviolet (UV) light.

3.2.2 *most probable number (MPN), n*—a statistical method for determining bacterial density based on the Poisson distribution.

3.2.3 *presence-absence, n*—a term used to indicate if enterococci is present in a water sample. It is a qualitative value, "yes" or "no" for reporting results.

3.2.4 *quanti-tray™, n*—a system for the quantification of enterococci. It consists of a sealer and trays which have multi-wells and can enumerate up to 2000 CFU/100 mL without dilution.

3.2.5 *snap pack, n*—a package containing Enterolert reagent for testing 100-mL sample either in the P/A format or quantitatively, that is, Quanti-Tray™ system).

4. Summary of Test Method [3]

4.1 This test method is used for the detection of enterococci, such as *E. faecium, E. faecalis* in drinking water, source water, recreational waters (marine water and fresh), and bottled water. When the reagent is added to the sample and incubated at 41 ± 0.5°C for 24 h, Enterolert can detect these bacteria at 1 CFU/100 mL. Fluorescence is produced when enterococci metabolizes the nutrient indicator. Enterolert can be used as a presence-absence test or for quantification (5-tube, 10-tube MPN, 15-tube serial dilution or the Quanti-Tray system).

5. Significance and Use

5.1 This test provides an easy and reliable method for the detection of enterococci in water within 24 h. For recreational water (fresh and marine) testing is performed to insure areas are safe for swimming. Enterolert also can be used for testing bottled water and drinking water.

6. Interferences

6.1 The presence of Bacillus spp. can interfere with the testing of marine water samples. To eliminate interference, a 1:10 dilution is required with sterile water (deionized or distilled).

7. Apparatus

7.1 *Ultraviolet Lamp*, 6-watt long wavelength (366 nm).
7.2 *41°C Incubator (± 0.5°C)*, air or water bath.
7.3 *Vessels*, sterile, nonfluorescent.
7.4 *Quanti-Tray Sealer*[4].
7.5 Quanti-Tray or Quanti-Tray 2000[4].

8. Reagents and Materials

8.1 *Purity of Water*—Unless otherwise indicated, references

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[1] This test method is under the jurisdiction of ASTM Committee D-19 on Water and is the direct responsibility of Subcommittee D19.24 on Microbiology.

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[2] *Annual Book of ASTM Standards*, Vol 11.01.

[3] This test method is based on Enterolert, a product of IDEXX Laboratories, Westbrook, ME 04092.

[4] Available from IDEXX Laboratories, One Idexx Dr., Westbrook, ME 04092.

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to water shall be understood to mean reagent water conforming to Specification D 1193, Type IV. Sterilize the water by either autoclaving or by sterile filtration (0.22 micron-filtered water).

8.2 *Enterolert Test Kit*[4].

9. Precautions

9.1 The analyst must observe the normal good laboratory practices and safety procedures required in a microbiology laboratory while preparing, using, and disposing of cultures, reagents and materials and while operating sterilization equipment and other equipment.

10. Sampling

10.1 Collect the sample as described in detail in the USEPA microbiological methods manual[5] and in accordance with Practices D 3370.

10.2 *Sample Storage Temperature and Handling Conditions*—Ice or refrigerate water samples at a temperature of 2 to 8°C during transit to the laboratory. Use insulated containers to ensure proper maintenance of storage temperatures. Take care that sample bottles are not totally immersed in water during transit or storage.

10.3 *Holding Time Limitations*—Examine samples, as soon as possible, after collection. Do not hold samples longer than 6 h between collection and initiation of analyses.

11. Quality Control Check

11.1 Check and record temperatures in incubators daily to insure temperature is within stated limits.

11.2 Quality control should be conducted on each new lot of Enterolert. See package insert for the recommended quality control procedure, which consists of the following protocol:

11.2.1 For each type of the American Type Culture Collection (ATCC) bacterial strain listed below, streak the culture onto labeled TSA or blood agar plates and incubate at 35°C for 18–24 h.

11.2.2 For each bacterial strain, touch a 1-µl loop to a colony and use it to inoculate a labeled test tube containing 5 mL of sterile deionized water. Close cap and shake thoroughly.

11.2.3 For each bacterial strain, take a 1-µl loop from the test tube and use it to inoculate a labeled vessel containing 100 mL.

11.2.4 Follow the Enterolert presence/absence steps listed above to test these controls. Compare the test results to the following expected results:

Control	ATTC No.	Expected Result
Enterococcus faecium	335667	Fluorescence
Serratia marcescens (g, −)	43862	No fluorescence
Aerococcus viridians (g, +)	10400	No fluorescence

12. Procedure

12.1 *Presence/Absence*—See package insert.

12.1.1 Samples should be brought to room temperature (18–30°C).

12.1.2 Carefully separate one snap pack from the strip.

12.1.3 Tap the snap pack to insure that all of the powder is towards the bottom of the pack.

12.1.4 Open the pack by snapping back the top of the score line. Do not touch the opening of pack.

12.1.5 Add the reagent to a 100-mL water sample, which is in a sterile, transparent, nonfluorescent vessel.

12.1.6 Aseptically cap and seal the vessel.

12.1.7 Shake until dissolved.

12.1.8 Incubate Enterolert for 24 h at 41 ± 0.5°C,

12.1.9 Read results at 24 h. If the sample is inadvertently incubated over 28 h without observation, the following guidelines apply: Lack of fluorescence after 28 h is a valid negative test. Fluorescence after 28 h is an invalid result.

12.1.10 Check for fluorescence by placing a 6-W 366-nm UV light within 5 in. of the sample in a dark environment. Be sure the light is facing away from your eyes and towards the vessel. If fluorescence is observed, the presence of enterococci is confirmed.

12.2 *MPN*—Quanti-tray enumeration test procedure for 100-mL sample (see package insert).

12.2.1 Follow steps 12.1.1-12.1.7.

12.2.2 Pour the reagent sample into the Quanti-Tray avoiding contact with the foil tab and seal the tray according to the Quanti-Tray package insert.

12.2.3 Incubate for 24 h at 41 ± 0.5°C.

12.2.4 Follow the same interpretation instructions from 12.1.9 through 12.1.10, and count the number of positive wells. Refer to the MPN table (see Table 1) provided with the Quanti-Tray to determine the CFU/100 mL.

12.3 *MPN*—5-tube × 20 mL, 10-tube × 10 mL and 15-tube serial dilution.

12.3.1 Follow 12.1.1-12.1.7.

12.3.2 sterile nonfluorescent tubes or transfer 20 mL of the reagent sample into five sterile nonfluorescent tubes.

12.3.3 Incubate for 24 h at 41 ± 0.5°C.

12.3.4 Follow 12.1.9 and 12.1.10 for interpretation.

12.3.5 Refer to the MPN tables (see Tables 2-4) to determine the CFU/100 mL.

13. Calculation

13.1 For P/A, there are no calculations. For quantification, refer to Quanti-Tray MPN tables and for the 5, 10, and 15 tube test results refer to the respective MPN tables.[6]

14. Report

14.1 Report as positive or negative for presence/absence testing.

14.2 Reporting of results is based on calculation of enterococci density determined from the appropriate MPN tables.

15. Precision and Bias [7]

15.1 *Precision*—A limited collaborative study was conducted. Nine technicians from three laboratories tested three different matrixes at three levels following Practice D 2777. Outliers were rejected in accordance with the statistical tests outlined in Practice D 2777. All data from one technician was rejected for recreational water-marine and single values were rejected for both recreational water-fresh at the low level and for recreational water-marine at the low level. The mean count, the overall standard deviation (St), and the single operator standard deviation (so), are indicated in Table 5.

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[5] Bordner, R.H., Winter, J.A., and Scarpino, P.V., Eds., *Microbiological Methods for Monitoring the Environment, Water, and Wastes*, EPA-600/8-78-017.

[6] *Standard Methods for the Examination of Water and Waste Water*, 19th Edition.

[7] Supporting data for this test method are available from ASTM Headquarters. Request RR: D 19-1167.

15.2 *Bias*—The mean value obtained for the samples (drinking water, recreational water fresh and marine) from the nine technicians for the low-, mid- and high-spiked samples all fall within the 95 % confidence interval (poisson distribution) of the actual values obtained from plating on blood agar.

15.3 Results of this collaborative study may not be typical of results for matrices other than those studied.

16. Keywords

16.1 bottled water; drinking water; enterococci; Enterolert; most probable number; presence-absence; Quanti-Tray; recreational water; source water

TABLE 1 51-Well Quanti-Tray® MPN Table

No. of Wells Giving Positive Reaction	MPN/100-mL Sample	95 % Confidence Limits	
		Lower	Upper
0	<1	0.0	3.7
1	1.0	0.3	5.6
2	2.0	0.6	7.3
3	3.1	1.1	9.0
4	4.2	1.7	10.7
5	5.3	2.3	12.3
6	6.4	3.0	13.9
7	7.5	3.7	15.5
8	8.7	4.5	17.1
9	9.9	5.3	18.8
10	11.1	6.1	20.5
11	12.4	7.0	22.1
12	13.7	7.9	23.9
13	15.0	8.8	25.7
14	16.4	9.8	27.5
15	17.8	10.8	29.4
16	19.2	11.9	31.3
17	20.7	13.0	33.3
18	22.2	14.1	35.2
19	23.8	15.3	37.3
20	25.4	16.5	39.4
21	27.1	17.7	41.6
22	28.8	19.0	43.9
23	30.6	20.4	46.3
24	32.4	21.8	48.7
25	34.4	23.3	51.2
26	36.4	24.7	53.9
27	38.4	26.4	56.6
28	40.6	28.0	59.5
29	42.9	29.7	62.5
30	45.3	31.5	65.6
31	47.8	33.4	69.0
32	50.4	35.4	72.5
33	53.1	37.5	76.2
34	56.0	39.7	80.1
35	59.1	42.0	84.4
36	62.4	44.6	88.8
37	65.9	47.2	93.7
38	69.7	50.0	99.0
39	73.8	53.1	104.8
40	78.2	56.4	111.2
41	83.1	59.9	118.3
42	88.5	63.9	126.2
43	94.5	68.2	135.4
44	101.3	73.1	146.0
45	109.1	78.6	158.7
46	118.4	85.0	174.5
47	129.8	92.7	195.0
48	144.5	102.3	224.1
49	165.2	115.2	272.2
50	200.5	135.8	387.6
51	>200.5	146.1	infinite

TABLE 2 IDEXX Quanti-Tray/2000 MPN Table (cfu/100 mL)

No. Large Wells Positive	No. Small Wells Positive																							
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.0	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.2
1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.3	16.4	17.4	18.5	19.5	20.6	21.6	22.6	23.7	24.8	25.8
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.4	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.2	26.3	27.4	28.5
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	15.9	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3
7	7.4	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.5	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8
10	11.0	12.1	13.2	14.3	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.7	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.4	40.7
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.1	42.4
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.4	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9
17	20.3	21.6	22.8	24.0	25.3	26.5	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.4	47.8	49.1	50.5	51.9
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0
20	24.9	26.2	27.5	28.8	30.1	31.4	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1
21	26.5	27.8	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.7	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.7
23	29.9	31.3	32.7	34.1	35.4	36.8	38.2	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.6	43.1	44.6	46.0	47.5	49.0	50.5	51.9	53.4	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8
25	33.5	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4
26	35.5	36.9	38.4	39.9	41.3	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2
27	37.4	38.9	40.4	41.9	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2
28	39.5	41.0	42.6	44.1	45.7	47.2	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.1	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3
29	41.6	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	68.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.8	74.7	76.5	78.3	80.2	82.1	84.0
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.1	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5
33	51.2	53.0	54.7	56.5	58.3	60.1	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.5	95.7
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	66.9	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.1
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2
39	69.9	72.2	74.4	76.6	78.9	81.3	83.6	86.0	88.4	90.9	93.3	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.5	117.4	120.3	123.2	126.1	129.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.7	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.2	127.3	130.5	133.7	137.0
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.3	138.8	142.3	145.9
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.3	126.7	130.1	133.6	137.1	140.8	144.5	148.3	152.2	156.1
43	87.6	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.1	159.4	163.8	168.2
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	158.5	163.1	167.8	172.7	177.7	182.9
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.3	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	177.9	183.5	189.2	195.1	201.2
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	166.9	172.5	178.2	184.2	190.4	196.8	203.5	210.5	217.8	225.4
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.3	214.2	222.4	231.0	240.0	249.5	259.5
48	123.9	128.4	133.1	137.9	143.0	148.3	153.9	159.7	165.8	172.2	178.9	186.0	193.5	201.4	209.8	218.7	228.2	238.2	248.9	260.2	272.3	285.1	298.7	313.0
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.9	248.1	261.3	275.5	290.9	307.6	325.5	344.8	365.4	387.3	410.6

TABLE 3 IDEXX Quanti-Tray/2000 MPN Table (cfu/100 mL)

	No. Small Wells Positive																							
No.	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
	25.3	26.3	27.4	28.4	29.5	30.5	31.5	32.6	33.6	34.7	35.7	36.8	37.8	38.9	39.9	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.5
	26.6	27.6	28.7	29.7	30.8	31.9	32.9	34.0	35.0	36.1	37.2	38.2	39.3	40.4	41.4	42.5	43.6	44.7	45.7	46.8	47.9	49.0	50.1	51.2
	27.9	29.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7	40.8	41.9	42.9	44.0	45.1	46.2	47.3	48.4	49.5	50.6	51.7	52.8
	29.3	30.3	31.4	32.5	33.6	34.7	35.7	36.8	37.9	39.0	40.1	41.2	42.3	43.4	44.5	45.6	46.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5
	30.7	31.7	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8	43.9	45.0	46.1	47.2	48.3	49.5	50.6	51.7	52.9	54.0	55.1	56.3
	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.8	41.0	42.1	43.2	44.3	45.5	46.6	47.7	48.9	50.0	51.2	52.3	53.5	54.6	55.8	56.9	58.1
	33.5	34.6	35.8	36.9	38.0	39.1	40.3	41.4	42.6	43.7	44.8	46.0	47.1	48.3	49.4	50.6	51.7	52.9	54.1	55.2	56.4	57.6	58.7	59.9
	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.8	50.0	51.2	52.3	53.5	54.7	55.9	57.1	58.2	59.4	60.6	61.8
	36.5	37.7	38.9	40.0	41.2	42.3	43.5	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.5	57.7	58.9	60.2	61.4	62.6	63.8
	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2	52.4	53.6	54.8	56.0	57.2	58.4	59.7	60.9	62.1	63.4	64.6	65.8
	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.6	51.8	53.0	54.2	55.5	56.7	57.9	59.2	60.4	61.6	62.9	64.2	65.4	66.7	67.9
	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.6	54.9	56.1	57.4	58.6	59.9	61.2	62.4	63.7	65.0	66.2	67.5	68.8	70.1
	43.1	44.3	45.6	46.8	48.1	49.3	50.5	51.8	53.1	54.3	55.6	56.8	58.1	59.4	60.7	61.9	63.2	64.5	65.8	67.1	68.4	69.7	71.0	72.3
	44.9	46.1	47.4	48.6	49.9	51.2	52.4	53.7	55.0	56.3	57.6	58.9	60.2	61.5	62.8	64.1	65.4	66.7	68.0	69.3	70.7	72.0	73.3	74.7
	46.7	48.0	49.3	50.5	51.8	53.1	54.4	55.7	57.0	58.3	59.6	60.9	62.3	63.6	64.9	66.3	67.6	68.9	70.3	71.6	73.0	74.4	75.7	77.1
	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1	64.5	65.8	67.2	68.5	69.9	71.3	72.6	74.0	75.4	76.8	78.2	79.6
	50.5	51.8	53.2	54.5	55.8	57.2	58.5	59.9	61.2	62.6	64.0	65.3	66.7	68.1	69.5	70.9	72.3	73.7	75.1	76.5	77.9	79.3	80.8	82.2
	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1	70.5	71.9	73.3	74.8	76.2	77.6	79.1	80.5	82.0	83.5	84.9
	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	78.8	80.3	81.8	83.3	84.8	86.3	87.8
	56.8	58.2	59.6	61.0	62.4	63.9	65.3	66.7	68.2	69.7	71.1	72.6	74.1	75.5	77.0	78.5	80.0	81.5	83.1	84.6	86.1	87.6	89.2	90.7
	59.0	60.4	61.9	63.3	64.8	66.3	67.7	69.2	70.7	72.2	73.7	75.2	76.7	78.2	79.8	81.3	82.8	84.4	85.9	87.5	89.1	90.6	92.2	93.8
	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	76.4	77.9	79.5	81.0	82.6	84.2	85.8	87.4	89.0	90.6	92.2	93.8	95.4	97.1
	63.7	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8	82.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5
	66.3	67.8	69.4	71.0	72.5	74.1	75.7	77.3	78.9	80.5	82.1	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	98.9	100.6	102.3	104.1
	68.9	70.5	72.1	73.7	75.3	77.0	78.6	80.2	81.9	83.6	85.2	86.9	88.6	90.3	92.0	93.8	95.5	97.2	99.0	100.7	102.5	104.3	106.1	107.9
	71.7	73.3	75.0	76.6	78.3	80.0	81.6	83.3	85.0	86.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	106.3	108.2	110.0	111.9
	74.6	76.3	78.0	79.7	81.4	83.1	84.8	86.6	88.4	90.1	91.9	93.7	95.5	97.3	99.2	101.0	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2
	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.5	97.4	99.3	101.2	103.1	105.0	106.9	108.8	110.8	112.7	114.7	116.7	118.7	120.7
	80.8	82.6	84.4	86.2	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6
	84.2	86.1	87.9	89.8	91.7	93.6	95.6	97.5	99.5	101.5	103.5	105.5	107.5	109.5	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8
	87.8	89.7	91.7	93.6	95.6	97.6	99.6	101.6	103.7	105.7	107.8	109.9	112.0	114.1	116.3	118.5	120.6	122.8	125.1	127.3	129.5	131.8	134.1	136.4
	91.6	93.6	95.6	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7	116.9	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5
	95.7	97.7	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.8	129.2	131.6	134.0	136.5	139.0	141.5	144.0	146.6	149.1
	100.0	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2	120.5	122.9	125.3	127.8	130.3	132.8	135.3	137.8	140.4	143.0	145.6	148.3	150.9	153.6	156.4
	104.7	107.0	109.3	111.7	114.0	116.4	118.9	121.3	123.8	126.3	128.8	131.4	134.0	136.6	139.2	141.9	144.6	147.3	150.1	152.9	155.7	158.6	161.5	164.4
	109.7	112.2	114.6	117.1	119.6	122.1	124.7	127.3	129.9	132.6	135.3	138.0	140.8	143.6	146.4	149.2	152.1	155.0	158.0	161.0	164.0	167.1	170.2	173.3
	115.2	117.8	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.5	142.4	145.3	148.3	151.3	154.3	157.3	160.4	163.6	166.8	170.0	173.3	176.6	179.9	183.3
	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.2	150.3	153.5	156.6	159.9	163.1	166.4	169.8	173.2	176.7	180.2	183.7	187.3	191.0	194.7
	127.9	130.8	133.8	136.8	139.9	143.0	146.1	149.3	152.6	155.9	159.2	162.6	166.1	169.6	173.2	176.8	180.4	184.2	188.0	191.8	195.7	199.6	203.7	207.7
	135.3	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1	176.9	180.7	184.7	188.6	192.7	196.8	201.0	205.3	209.6	214.0	218.5	223.0
	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173.0	177.0	181.1	185.2	189.4	193.7	198.1	202.5	207.0	211.7	216.4	221.1	226.0	231.0	236.0	241.1
	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.4	185.8	190.3	194.8	199.5	204.2	209.1	214.0	219.0	224.2	229.4	234.8	240.2	245.8	251.5	257.2	263.1
	164.3	168.6	172.9	177.3	181.9	186.5	191.3	196.1	201.1	206.2	211.4	216.7	222.1	227.7	233.4	239.2	245.2	251.3	257.5	263.8	270.3	276.9	283.6	290.5
	177.5	182.3	187.3	192.4	197.6	202.9	208.4	214.0	219.8	225.8	231.8	238.1	244.5	251.0	257.7	264.6	271.7	278.9	286.3	293.8	301.5	309.4	317.4	325.6
	193.6	199.3	205.0	211.0	217.2	223.5	230.0	236.7	243.6	250.7	258.1	265.6	273.3	281.2	289.4	297.8	306.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4
	214.1	220.9	227.9	235.1	242.7	250.4	258.4	266.7	275.3	284.1	293.2	302.6	312.3	322.3	332.5	343.0	353.8	364.9	376.2	387.9	399.8	412.0	424.5	437.4
	241.5	250.0	258.9	268.2	277.8	287.7	298.1	308.8	319.9	331.4	343.3	355.5	368.1	381.1	394.5	408.3	422.5	437.0	452.0	467.4	483.3	499.5	516.3	533.5
	280.9	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.8	436.6	454.1	472.1	490.7	509.9	529.8	550.4	571.7	593.8	616.7	640.5	665.3	691.0
	344.1	360.9	378.4	396.8	416.0	436.0	456.9	478.6	501.2	524.7	549.2	574.8	601.5	629.4	658.6	689.3	721.5	755.5	791.5	829.7	870.4	913.9	960.6	1011.1
	461.1	488.4	517.2	547.5	579.4	613.1	648.8	686.7	727.0	770.1	816.4	866.4	920.8	980.4	1046.2	1119.9	1203.3	1299.7	1413.6	1553.1	1732.9	1986.3	2419.2	>2419.2

TABLE 4 MPN Index and 95 % Confidence Limits for Various Combinations of Positive Results When Five Tubes are Used/Dilution (10 mL, 1.0 mL, 0.1 mL)[A]

Combination of Positives	MPN Index/100 mL	95 % Confidence Limits	
		Lower	Upper
0-0-0	<2	—	—
0-0-1	2	1.0	10
0-1-0	2	1.0	10
0-2-0	4	1.0	13
1-0-0	2	1.0	11
1-0-1	4	1.0	15
1-1-0	4	1.0	15
1-1-1	6	2.0	18
1-2-0	6	2.0	18
2-0-0	4	1.0	17
2-0-1	7	2.0	20
2-1-0	7	2.0	21
2-1-1	9	3.0	24
2-2-0	9	3.0	25
2-3-0	12	5.0	29
3-0-0	8	3.0	24
3-0-1	11	4.0	29
3-1-0	11	4.0	29
3-1-1	14	6.0	35
3-2-0	14	6.0	35
3-2-1	17	7.0	40
4-0-0	13	5.0	38
4-0-1	17	7.0	45
4-1-0	17	7.0	46
4-1-1	21	9.0	55
4-1-2	26	12	63
4-2-0	22	9.0	56
4-2-1	26	12	65
4-3-0	27	12	67
4-3-1	33	15	77
4-4-0	34	16	80
5-0-0	23	9.0	86
5-0-1	30	10	110
5-0-2	40	20	140
5-1-0	30	10	120
5-1-1	50	20	150
5-1-2	60	30	180
5-2-0	50	20	170
5-2-1	70	30	210
5-2-2	90	40	250
5-3-0	80	30	250
5-3-1	110	40	300
5-3-2	140	60	360
5-3-3	170	80	410
5-4-0	130	50	390
5-4-1	170	70	480
5-4-2	220	100	580
5-4-3	280	120	690
5-4-4	350	160	820
5-5-0	240	100	940
5-5-1	300	100	1300
5-5-2	500	200	2000
5-5-3	900	300	2900
5-5-4	1600	600	5300
5-5-5	≥1600	—	—

[A] Based on *Standard Methods for the Examination of Water and Wastewater*, 19th ed.

TABLE 5 Mean Count, Overall Standard Deviation and Single Operator Standard Deviation

NOTE 1—All calculations were made from the statistical summary given as Table One in the study file

Summary Table	Low Level				Middle Level				High Level			
Background Matrix	n	X MPN/100 mL	St %	So %	n	X MPN/100 mL	St %	So %	n	X MPN/100 mL	St %	So %
Drinking water	9	9.1	27.7	24.2	9	26.2	15.1	15	9	61.0	10.2	9.5
Rec. water Fresh	9	9.4[A]	30.2[A]	23.4[A]	9	29.1	14.1	6.9	9	64.3	10.6	11
Rec. water Marine	8	7.9[A]	25.2[A]	17.7[A]	8	29.4	17.4	13	8	67.5	8.0	7.6

[A] One value rejected to make this estimate.

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